

✓ lines 19-20, please delete "(Figure 8A)." and replace therewith --(8A and 8B),--;

✓ line 21, please delete "(Figure 8B)." and replace therewith --(Figure 8C). --.

✓ At page 414, line 18, please delete "Figure 10A)." and replace therewith -- Figures 10A, 10B, 10C, 10D, and 10E).--;

✓ line 23, please delete "Figure 10B)." and replace therewith --Figures 10F and 10G).--.

✓ At page 416, line 20, please delete "(Figure 11B)." and replace therewith -- (Figures 11B and 11C).--.

✓ At page 417, line 12, please delete "(Figure 11C)." and replace therewith -- (Figures 11D, 11E and 11F).--.

In the Drawings:

Please replace the originally filed Figures 1A-B, 2A-B, 3, 4A-C, 5A-B, 6, 7a-b, 8a-b, 9a-b, 10a-b and 11a-c with the Formal Drawings of Figures 1A-B, 2A-D, 3, 4A-C, 5A-B, 7A-1-A-2 and 7B, 8A-C, 9A-B, 10A-G and 11A-F submitted herewith.

REMARKS

Amendments to the Specification

The majority of the amendments being made in the specification merely correct the references to the Figures such that they conform with the figure labels of the Formal Drawings. All amendments that do more than correct reference to Figures are discussed in more detail below.

Grammatical errors have been corrected by the amendment of the word "shows" on pages 24 and 26, lines 19 and 20 respectively, to "show".

We have removed reference to Figures 12A and 12B on page 83, line 6 because no Figure 12A or Figure 12B was submitted with the application.

No new matter is introduced by these amendments.

SEP 14 2000

Amendments to the Drawings

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In Figure 7A (Formal Drawing 7A-1), the following minor changes have been made: 1) the line demarcating the transmembrane region has been shortened such that it extends only over the L at position 72 and no further; 2) the amino acid number designation "77", at the end of the first line of LT-alpha sequence has been changed to "76"; and 3) the "G" at position 197 of Neutrokin-alpha sequence was changed from being white on black to being black on white. Copies of the original drawing, in both its informal and formal form, are attached with these changes marked in red.

The amendment of the length of the line demarcating the transmembrane region brings the drawing into conformity with the description of the transmembrane region as it is defined in the specification in several locations. See for example page 10, lines 20-23 where the transmembrane region is defined as amino acids 47 to 72 of the Neutrokin-alpha polypeptide. Thus, this amendment introduces no new matter and is fully supported by the specification.

The amendment of the amino acid number designation corrects the numbering of the sequence such that it agrees with the numbering of the sequence of Lymphotoxin-alpha given in Gray, *Nature* 312,721-724 (1984). The Gray *et al.* *Nature* article is cited on page 412, line 15 of the specification in reference to Figure 7A. GenBank Reports for Accession Numbers CAA25649 and P01374 are submitted in support of this amendment. CAA25649 is the original GenBank submission for the Gray *et al.* article. Because the Gray GenBank report does not identify the lymphotoxin (LT) as LT-alpha, GenBank report P01374, which also cites the Gray *et al.* reference, is also included. Thus, this amendment introduces no new matter and is fully supported by the specification.

The shading of certain amino acid residues in Figure 7 indicates which amino acid residues match the consensus sequence. The amendment of the "G" at position 197 of Neutrokin-alpha sequence corrects the shading because a serine (S), and not a glycine (G) residue is the consensus residue at this position. Support for this amendment is found in Figure 2B (Formal Drawing Figure 2C) where the G at position 197 of the Neutrokin alpha polypeptide is not shaded. Thus, this amendment introduces no new matter and is fully supported by the specification.

CONCLUSION

Applicants respectfully request that the amendments and remarks above be entered and made of record in the file history of the instant application.

Respectfully submitted,

Dated: July 28, 2000



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Figure 7

a.

leutokine-

alpha M DD STEREQSRLTSCCLKKREEMKLKECVSILPRKESPSVRS 41
 SKD G K L L A A T L L L A L L S C C L T V V S F Y Q V A A L Q G D L A S L R A E 82
 L Q G H H A E K L P A G A G A P K A G L E E A P A V T A G L K I F E P P A P G E G 123

Transmembrane Region

DELETE PORTION
OF LINE IN BRACKETS

NSSQNSRNKRAVQGPEETVT QDC [] SEPTIQKGSY 164
 April HS [] VPINA SK-DDSDV 134
 TNF [] A H V V A N D Q A E G Q - - - - - 102
 LT α [] P A A H [] G [] SKQNS - - - - - 77

ECHANGE
"77" to
--76--

A' B' B C
 F P W L S - - - - F K A G S A E E K E N K I [] K E T G Y F [] 200
 E M Q P A - - - - R H R G Q A Q G Y G V R I Q D A G V L L [] 170
 - L Q W N R R A N A L A N C V E R D - - Q Q V V S E G L L [] 139
 - L L V R A N T D R F Q D F S L S N - - S [] V E T S G I [] V 114

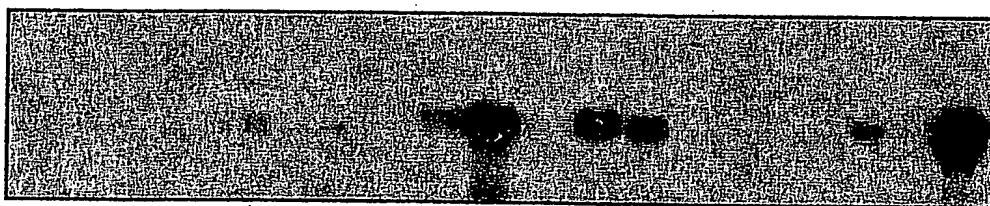
"G" at
POSITION
197 SHOULD
BE BLACK
TEXT ON
White
BACKGROUND

D E
 Y T D K [] - - - - A [] I Q R K K V H V G D E L S L V [] N 237
 Q D V F - - - - M Q V V S R E - - - - G G R Q E T [] R S 201
 K G O G C P - - - - S T V L T H T I S R I A V S Q T K V N L L S A I K S 176
 S G A A S P K A S S P Y A E E Q L H S S P F H V P L L S [] V 155

F G
 E - - T L E - - - - N S C V S A G I A K E G E Q L A T E N A 268
 S H P D R A - - - - N S C V S A G I A K E G E Q L A T E N A 234
 C Q R E T E G A E A K E P I V L G G V F O L K G R A E N R P D Y 217
 Y P - - - - G L Q E W L H M H G A A F O L T Q G Q T H T D G I P H 190

H
 Q I S D G D V [] G A L K L 285
 K L N S S H G [] L G F V 250
 D F A E S G Q V Y [] I I A 233
 V L S - - S T V [] G F A 205

b.



- 4.4 kb

- 2.4 kb

HL-60
 HeLa
 K-562
 MOLT-4
 Raji
 SW480
 Spleen
 Lymph Node
 Thymus
 PBL
 Bone Marrow
 Fetal liver
 Heart
 Brain
 Placenta
 Lung
 Liver
 S. Muscle
 Kidney
 Pancreas

Neutrokin-

Alpha MDDSTEREQSRLTSC LKREEMKLKECVSILPRKESPSVRS 41

DELETE PORTION OF
LINE IN BRACKETS

Transmembrane Region

SKDGKLLAATLLALLSCCLTVVSFYQVAALQGDLASLRAE 82

LQGHHAELPAGAGAPKAGLEEEAPAVTAGLKIFEPAPGEG 123

A

NSSQNSRNKRAVQGP EETVTQDC [QLIADSEI] PTIQKGSY 164
Apr11 HSVLHLVPINATSK DDSDV 134
TNF KPVAHVVAANPQAEGQ - - - - 102
LTα KPAAHLIGDPPSKQNS - - - - 77

Change "77"
to --76--

A'

FVPM LLS - - - - FKRGSA
EVMMPQA - - - - LRRGRGL
- LQWENRRANA - - - - LLAANGVEL
- LLWRANTDRA - - - - FLQDGFSL

B'

EEKEEQAQGYGVRIQDAGVYFF
QAGYGVRIQDAGVYFF
ANGVELLQDGFSL

B

NKI LMKETG YFF
NKG YGVRIQDAGVYFF
NQLVVP TSGIYFVYSQV 114

C

ELYGQVL 200
LLYSQVL 170
LIYSQVL 139
FVYSQV 114

"G" AT POSITION
197 Should be
black text on
white background

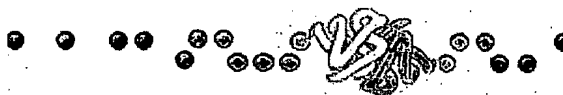
D

YTDKTY - - - - AMGHLIQ
FQDVTF - - - - TMGQVVS
FKGQGC - - - - STHVL
FSGKAYSPKATSSP L

E

FGDELSLVTLFRCIQNMP 237
GGRQETLFRCI RSM 201
TISR IAVSYQTKVNLLSAIKSP 176
HEVQLFESSQYPFHVPL LSSQKMV 155

FIG.7A-1



Search for

☒ 1 : GI = "34445" [GenPept]

lymphotoxin precursor [Homo...]

[Related Articles](#). [Protein](#), [Nucleotide](#)

LOCUS CAA25649 205 aa PRI 12-JUL-1993
 DEFINITION lymphotoxin precursor [Homo sapiens].
 ACCESSION CAA25649
 PID g34445
 VERSION CAA25649.1 GI:34445
 DBSOURCE embl locus HSLYTR, accession [X01393.1](#)
 KEYWORDS
 SOURCE human.
 ORGANISM [Homo sapiens](#)
 Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
 Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (residues 1 to 205)
 AUTHORS Gray,P.W., Aggarwal,B.B., Benton,C.V., Bringman,T.S., Henzel,W.J.,
 Jarrett,J.A., Leung,D.W., Moffat,B., Ng,P., Svedersky,L.P.,
 Palladino,M.A. and Nedwin,G.E.
 TITLE Cloning and expression of cDNA for human lymphotoxin, a lymphokine
 with tumour necrosis activity
 JOURNAL Nature 312 (5996), 721-724 (1984)
 MEDLINE [85086243](#)
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 /product="lymphotoxin"
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 /coded_by="X01393.1:80..697"
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 121 spkatssply lahevqlfss qypfhvplls sqkmvypglq-epwlhsmhyg aafqltqgdq
 181 lsthtdgiph lvlsptvff gafal
 //

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Search for

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1 : GI = "135940" [GenPept]

LYMPHOTOXIN-ALPHA PRECURSOR...

Related Articles, Protein

LOCUS TNFB HUMAN 205 aa PRI 01-NOV-1997

DEFINITION LYMPHOTOXIN-ALPHA PRECURSOR (LT-ALPHA) (TNF-BETA).

ACCESSION P01374

PID g135940

VERSION P01374 GI:135940

DBSOURCE swissprot: locus TNFB_HUMAN, accession P01374;
class: standard.
created: Jul 21, 1986.
sequence updated: Mar 1, 1989.
annotation updated: Nov 1, 1997.
xrefs: gi: 34444, gi: 34445, gi: 37215, gi: 312411, gi: 219913, gi:
219914, gi: 339739, gi: 339740, gi: 219911, gi: 219912, gi: 339742,
gi: 339743, gi: 37211, gi: 37213, gi: 412160, gi: 412161, gi:
1070570
xrefs (non-sequence databases): MIM 153440, PFAM PF00229, PROSITE
PS00251, PROSITE PS50049

KEYWORDS Cytokine; Glycoprotein; Cytotoxin; Signal; Polymorphism.

SOURCE human.

ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
Eutheria; Primates; Catarrhini; Hominidae; Homo.

REFERENCE 1 (residues 1 to 205)

AUTHORS NEDOSPASOV,S.A., SHAKHOV,A.N., TURETSKAYA,R.L., METT,V.A.,
AZIZOV,M.M., GEORGIEV,G.P., KOROBKO,V.G., DOBRYNIN,V.N.,
FILIPPOV,S.A., BYSTROV,N.S., BOLDYREVA,E.F., CHUVPILO,S.A.,
CHUMAKOV,A.M., SHINGAROVA,L.N. and OVCHINNIKOV,Y.A.

TITLE Tandem arrangement of genes coding for tumor necrosis factor
(TNF-alpha) and lymphotoxin (TNF-beta) in the human genome

JOURNAL Cold Spring Harb. Symp. Quant. Biol. 51 Pt 1, 611-624 (1986)

MEDLINE 87217060

REMARK SEQUENCE FROM N.A.

REFERENCE 2 (residues 1 to 205)

AUTHORS Nedwin,G.E., Jarrett-Nedwin,J., Smith,D.H., Naylor,S.L.,
Sakaguchi,A.Y., Goeddel,D.V. and Gray,P.W.

TITLE Structure and chromosomal localization of the human lymphotoxin
gene

JOURNAL J. Cell. Biochem. 29 (3), 171-181 (1985)

MEDLINE 86086150

REMARK SEQUENCE FROM N.A.

REFERENCE 3 (residues 1 to 205)

AUTHORS Kobayashi,Y., Miyamoto,D., Asada,M., Obinata,M. and Osawa,T.

TITLE Cloning and expression of human lymphotoxin mRNA derived from a
human T cell hybridoma

JOURNAL J. Biochem. 100 (3), 727-733 (1986)

MEDLINE 87057135

REMARK SEQUENCE FROM N.A.

REFERENCE 4 (residues 1 to 205)

AUTHORS GRAY,P.W., AGGARWAL,B.B., BENTON,C.V., BRINGMAN,T.S., HENZEL,W.J.,
JARRETT,J.A., LEUNG,D.W., MOFFAT,B., NG,P., SVEDERSKY,L.P.,
PALLADINO,M.A. and NEDWIN,G.E.

TITLE Cloning and expression of cDNA for human lymphotoxin, a lymphokine
with tumour necrosis activity

JOURNAL Nature 312 (5996), 721-724 (1984)

MEDLINE 85086243

REMARK SEQUENCE FROM N.A.

REFERENCE 5 (residues 1 to 205)

AUTHORS Matsuyama,N., Okawa,N., Tsukii,Y., Endo,T. and Kaji,A.

TITLE Nucleotide sequence of a cDNA encoding human tumor necrosis factor
beta from B lymphoblastoid cell RPMI 1788

JOURNAL FEBS Lett. 302 (2), 141-144 (1992)

MEDLINE 92339500
 REMARK SEQUENCE FROM N.A.
 REFERENCE 6 (residues 1 to 205)
 AUTHORS IRIS,F.J.M., BOUGUELERET,L., PRIEUR,S., CATERINA,D., PRIMAS,G.,
 PERROT,V., JURKA,J., RODRIGUEZ-TOME,P., CLAVERIE,J.-M., DAUSSET,J.
 and COHEN,D.
 TITLE Dense Alu clustering and a potential new member of the NF kappa B
 family within a 90 kilobase HLA class III segment
 JOURNAL Nat. Genet. 3 (2), 137-145 (1993)
 MEDLINE 93272029
 REMARK SEQUENCE FROM N.A.
 REFERENCE 7 (residues 1 to 205)
 AUTHORS Voigt,C.G., Maurer-Fogy,I. and Adolf,G.R.
 TITLE Natural human tumor necrosis factor beta (lymphotoxin). Variable
 O-glycosylation at Thr7, proteolytic processing, and allelic
 variation
 JOURNAL FEBS Lett. 314 (1), 85-88 (1992)
 MEDLINE 93083656
 REMARK PARTIAL SEQUENCE, CARBOHYDRATE-BINDING SITES, AND VARIANT.
 REFERENCE 8 (residues 1 to 205)
 AUTHORS Eck,M.J., Ultsch,M., Rinderknecht,E., de Vos,A.M. and Sprang,S.R.
 TITLE The structure of human lymphotoxin (tumor necrosis factor-beta) at
 1.9-A resolution
 JOURNAL J. Biol. Chem. 267 (4), 2119-2122 (1992)
 MEDLINE 92129275
 REMARK X-RAY CRYSTALLOGRAPHY (1.9 ANGSTROMS).
 REFERENCE 9 (residues 1 to 205)
 AUTHORS Abraham,L.J., Du,D.C., Zahedi,K., Dawkins,R.L. and Whitehead,A.S.
 TITLE Haplotypic polymorphisms of the TNFB gene
 JOURNAL Immunogenetics 33 (1), 50-53 (1991)
 MEDLINE 91139175
 REMARK VARIANT ALLELE 8.1.
 COMMENT

 This SWISS-PROT entry is copyright. It is produced through a
 collaboration between the Swiss Institute of Bioinformatics and
 the EMBL outstation - the European Bioinformatics Institute.
 The original entry is available from <http://www.expasy.ch/sprot>
 and <http://www.ebi.ac.uk/sprot>

[FUNCTION] LYMPHOTOXIN IS A CYTOKINE PRODUCED BY LYMPHOCYTES WHICH
 IS CYTOTOXIC FOR A WIDE RANGE OF TUMOR CELLS IN VITRO AND IN VIVO.
 [SUBUNIT] HETEROTRIMER OF EITHER TWO BETA AND ONE ALPHA SUBUNITS OR
 (LESS PREVALENT) TWO ALPHA AND ONE BETA SUBUNITS.
 [SUBCELLULAR LOCATION] SECRETED.
 [SIMILARITY] BELONGS TO THE TUMOR NECROSIS FACTOR FAMILY.

FEATURES Location/Qualifiers
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 /db_xref="taxon:9606"
 Region 1..34
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 Protein 1..205
 /product="LYMPHOTOXIN-ALPHA PRECURSOR"
 Region 13
 /region_name="Conflict"
 /note="C -> R (IN REF. 6)."
 Region 35..205
 /region_name="Mature chain"
 /note="LYMPHOTOXIN-ALPHA."
 Site 41
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 /note="PARTIAL."
 Region 60
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 /note="T -> N. /FTid=VAR_007511."
 Site 96
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 Region 125
 /region_name="Variant"
 /note="T -> P (IN ALLELE 8.1). /FTid=VAR_007512."

ORIGIN

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